

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
DEPARTMENT OF PESTICIDE REGULATION  
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA  
THIACLOPRID

Chemical Code # 5888, Tolerance # 52966  
SB 950 # NA.

23 November 2004  
Revised: May 20, 2005

I. DATA GAP STATUS

Combined, rat::	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, possible adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

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Toxicology one-liners are attached.

All record numbers through 216850 were examined.

\*\* indicates an acceptable study.

**Bold face** indicates a possible adverse effect.

File name: T205056A.wpd

Prepared by Green, 10/5/04; updated by Leung, 11/23/04

Revised, Moore, 5/20/05

## II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

## COMBINED, RAT

**\*\*52966-0071 209535**, "YRC 2894, Combined Chronic Toxicity/Carcinogenicity Study in Wistar Rats, Dietary Administration Over 2 Years", (E.M. Bomhard, *et al.*, Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No. 27480, Bayer Report No. 108359, 13 May 1998). 60 Wistar Hsd Cpb:WU rats per sex per group received YRC 2894 (96.8% thiacloprid) in the diet at 0, 25, 50, 500, and 1000 ppm for 107 weeks. 10 per sex per group were necropsied after 1 year of treatment. Mean daily YRC 2894 intake was 1.2, 2.5, 25.2, and 51.7 mg/kg of bodyweight in males and 1.6, 3.3, 33.5, and 69.1 mg/kg of bodyweight in females at 25, 50, 500, and 1000 ppm respectively. Food consumption was reduced ( $p \leq 0.01$ ) at 500 and 1000 ppm in males and females. The effect was more transient in males. Group mean bodyweight was decreased ( $p \leq 0.01$ ) for both sexes at 1000 ppm (Males, 12%, Females, 21%) and for females at 500 ppm (15%).

YRC 2894 induced phase I and II enzymes in liver homogenates of males and females at the interim necropsy. Phase I enzymes 7-ethoxycoumarin-deethylase (ECOD) and aldrin-epoxidase (ALD) were induced dose-dependently ( $p \leq 0.01$ ) from 25 ppm (up to a factor of 2 (ALD) to 4 (ECOD) times the control value at 1000 ppm). 7-ethoxyresorufin-deethylase (EROD) activity was not altered by treatment. Activities of phase II enzymes glutathione S-transferase (GS-T) and UDP-glucuronyl-transferase (GLU-T) were induced ( $p \leq 0.01$ ) (1.5x and 1.9x respectively) at 500 and 1000 ppm. Epoxide hydrolase (EH) was induced 1.5x at 25 and 50 ppm ( $p \leq 0.05$ ), 2.5x at 500 ppm ( $p \leq 0.01$ ) and 3x at 1000 ppm in females and 2x for males at 1000 ppm.

Thyroid stimulating hormone (TSH) was increased (ns) for males at 1000 ppm at week 26, 53, 78, and 105. Significant increases ( $p \leq 0.01$ ) in TSH were noted for high dose females at weeks 26 and 105.

Retinal atrophy was significantly increased in females at 50 ( $p \leq 0.05$ ), 500 ( $p \leq 0.05$ ), and 1000 ppm ( $p \leq 0.01$ ). Lens degeneration was also significantly increased ( $p \leq 0.01$ ) for females at 500 and 1000 ppm.

At terminal necropsy, in liver, centrilobular hypertrophy and cytoplasmic change were significantly increased ( $p \leq 0.01$ ) for males at 50 ppm and for both sexes at 500 and 1000 ppm. Mixed eosinophilic-clear cell foci were significantly increased in males at 50 ppm ( $p \leq 0.05$ ) and above ( $p \leq 0.01$ ) and in females at 1000 ppm ( $p \leq 0.01$ ). Increased hepatocellular hypertrophy was also noted for both sexes at 500 and 1000 ppm at the interim necropsy. Follicular epithelium hypertrophy was significantly increased at 50 ppm ( $p \leq 0.05$ ) in males and for both sexes ( $p \leq 0.01$ ) at 500 ppm and 1000 ppm at terminal necropsy. Increases in colloid alteration ( $p \leq 0.01$ ) and pigment in the follicular epithelium ( $p \leq 0.01$ /males and  $p \leq 0.05$ /females) were recorded at 500 and 1000 ppm.

Dose-dependent increases in neoplasms were noted at final necropsy. Thyroid follicular cell adenomas were increased in males at 500 ppm (0/50 for controls vs 5/50,  $p \leq 0.05$ ) and 1000 ppm (0/50 for controls vs 8/50,  $p \leq 0.01$ ). A slight increase (ns) was also noted for females at 1000 ppm (0/50 for controls vs 2/50). Additionally, uterine adenocarcinomas were increased at 500 and 1000 ppm (14/50 (28%) and 18/50 (36%) respectively vs 6/50 (12%) for controls). The incidence was above historical control values and significant ( $p \leq 0.01$ ) in the Trend test. Chronic NOEL = 25 ppm (centrilobular hypertrophy, cytoplasmic change, mixed eosinophilic-clear cell foci, and induction of Phase I and II enzymes in liver and thyroid follicular epithelium hypertrophy at 50 ppm in males). Possible adverse effects: dose-dependent increases in uterine adenocarcinomas in females and thyroid follicular cell adenomas in males. The changes may have resulted

secondary to the effect (dose-dependent) of YRC 2894 on the liver where, data suggest, induction of Phase I and II enzymes may alter the hormonal balance/pathways for estrogen and thyroid hormones. Acceptable. (Green and Gee, 8/24/04).

#### CHRONIC TOXICITY, DOG

**\*\*52966-0052** 209516, "YRC 2894, Chronic Toxicity Study in Beagle Dogs (52-Week Feeding Study)", (H. Wetzig and V. Geiss, Bayer AG, Department of Toxicology, Wuppertal, Germany, Laboratory Report No. 27563, Bayer 108501.5 June 1998). 4 Beagle dogs per sex per group received YRC 2894 (96.8% to 97.1% thiacloprid) in the diet at 0 (basal diet), 40, 100, 250, and 1000 ppm for 52 weeks. Additionally, 3 males per group received test diet at 0, 100, and 1000 ppm for 26 weeks. Group mean YRC 2894 intakes for both sexes in part 1 were 1.41, 3.44, 8.58, and 34.11 mg/kg/day at 40, 100, 250, and 1000 ppm respectively for weeks 4 through 52. For males in part 2, values were 3.23 and 32.21 mg/kg/day at 100 and 1000 ppm respectively for weeks 4 through 26. No treatment related changes on clinical signs, bodyweights, hematology, urinalysis, and ophthalmology were indicated. Slightly reduced food consumption was recorded occasionally for 1000 ppm females. Group mean absolute and relative prostate weights were increased in part 1 males at necropsy (52 weeks). The size of the prostate was also increased in these animals. Histopathology revealed no morphological changes. No prostatic differences were noted for part 2 males at the 26-week necropsy. Group mean absolute (389.3 g at 0 ppm vs 405.7 g at 1000 ppm) and relative liver weights (35.63 at 0 ppm vs 37.70 at 1000 ppm) were increased for part 2 high dose males at necropsy. Additionally, histopathology revealed an increase in hepatocellular cytoplasmic change in part 2 males at 1000 ppm at necropsy (26 weeks). These liver changes were not observed in part 1 animals at the 52 week sacrifice. Chronic NOEL = 250 ppm (8.58 mg/kg/day) based on liver changes. Acceptable. (Green and Gee, 8/23/04).

**\*\*52966-0051** 209515, "Subchronic Toxicity Study in Beagle Dogs (Feeding Study for About 15 Weeks)", (H. Wetzig and M. Rinke, Bayer AG, Institute for Toxicology, Wuppertal, Germany, Study No. T 0 058 331, Bayer Report No. 108350, 8 May 1998). 4 Beagle dogs per sex per group received YRC 2894 (96.8% thiacloprid) in the diet at 0 (basal diet), 250, 1000, and 2000 ppm for 15 weeks. High dose animals received treated feed for 13 weeks (days 1-4 at 4000 ppm, days 5-14 at 0, and day 15 to necropsy at 2000 ppm). Dosage was reduced due to vomiting, reduced feed intake, and refusal to eat. The group mean (both sexes) intakes of YRC 2894 during weeks 1 through 15 were 8.7, 34.7, and 66.7 mg/kg/day at 250, 1000, and 2000 ppm respectively. Slight reductions (NS) in food consumption (both sexes) and male bodyweight were recorded at the high dose level. Thyroxine (T4) was decreased at the mid and high dose levels. Absolute and relative prostate weights were increased at the mid and high dose levels and absolute and relative liver weights were higher in all treated groups (both sexes) relative to controls. Histopathology revealed slight to moderate hypertrophy of the prostate in all males at the mid and high dose levels. Additionally, at the high dose level, an increase in the number of degenerated spermatocytes was noted in the testes (control: 1/4 vs high dose: 2/4) and epididymides (control: 1/4 vs high dose: 4/4). Leydig cells appeared slightly more prominent at the high dose level (control: 1/4 vs high dose: 3/4). The significance of these changes is unclear given the wide variation in severity and incidence found in young mature dogs. Peak mean plasma levels (6 hours post-treatment) of YRC 2894 at week 14 indicated a high absorption rate (2, 6, and 14 µg/ml at the low, mid, and high dose levels respectively). NOEL = 250 ppm (8.7 mg/kg/day) based on increased prostate weight and hypertrophy. **Possible adverse effect:** prostate enlargement (hypertrophy). Acceptable. (Green and Gee, 8/19/04)

52966-0080 209548, "YRC 2894, Subacute Toxicity Study in Beagle Dogs (Dose Range Finding Study by Feed Admixture Over at Least 10 Weeks)", (H. Wetzig and V. Geiß, Bayer AG, Institute for Toxicology, Wuppertal, Germany, Study No. T 8 055 594, Report No. 27177 A, 11 February 1999). 2 Beagle dogs per sex received YRC 2894 (98.6% thiacloprid) in the diet at 0 (basal diet), 100, 300, 1000 ppm (increased to 1250 ppm from day 19, to 1600 ppm from day 26, and to 2500 ppm from day 38) for up to 10 weeks. An additional group received 2500 ppm from

day 38 through 66. The group mean YRC 2894 consumption for both sexes was 3.3, 9.6, 80.0, and 65.7 mg/kg/day at 100, 300, 1000 - 2500, and the additional 2500 ppm group respectively at week 9. Reduced food consumption and bodyweight were observed in females of the added 2500 ppm group during the 4 week treatment period. Urea and creatinine values were increased at 1000-2500 and 2500 ppm. Alanine aminotransferase values were slightly increased for males in the 2500 ppm group. Thyroxine (T4) was reduced and triiodothyronine (T3) was increased in 2500 ppm females. Marginal changes were identified in liver enzymes. O-Demethylase values were slightly increased in 2500 ppm group animals. 7-ethoxycoumarin deethylase (ECOD), epoxide hydrolase (EH), and glutathione-S--transferase (GS-T) were slightly increased in the 1000-2500 and 2500 ppm females. Absolute and relative prostate weights were increased for 1000-2500 and 2500 ppm males. The increase was greater at 1000-2500 ppm probably due to longer time on treatment. Microscopy revealed slight cytoplasmic change in liver cells of 1 female at 1000-2500 ppm and 1 per sex at added 2500 ppm. NOEL = 300 ppm (9.6 mg/kg/day) based on increased prostate weights, liver histopathology, and clinical chemistry. Not a guideline study. Supplemental. (Green and Gee, 10/21/04).

### ONCOGENICITY, MOUSE

**\*\*52966-0072 209536**, "YRC 2894, Oncogenicity Study in B6C3F1-Mice. Administration in the Food Over 2 Years.", (U. Wirtzinger and V. Geiss, Institute of Toxicology, Bayer AG, Wuppertal, Germany, Bayer Report No. 108358, 26 February 1998). Fifty B6C3F1 mice per sex per group received YRC 2894 (96.8% thiacloprid) in the diet at 0 (basal diet + 1% peanut oil), 30, 1250, and 2500 ppm for 2 years. Additionally, satellite groups of 10 per sex received 0 and 2500 ppm for 1 year followed by necropsy. Group mean daily test compound intakes were 5.7 and 10.9; 234.1 and 475.3; and 546.4 and 872.5 mg/kg bodyweight per day for males and females at 30, 1250, and 2500 ppm respectively. Group mean male bodyweight was reduced at 2500 ppm (sporadic bodyweight decreases were also noted for females in the initial weeks of the study at the high dose level). Leukocyte counts were significantly increased ( $p \leq 0.01$ ) for males at 1250 and 2500 ppm. Significant increases ( $p \leq 0.01$ ) in group mean relative liver weights were recorded for high dose interim and terminal sacrifice animals and at the mid dose after 2 years. Absolute and relative adrenal weights were increased ( $p \leq 0.01$ ) for high dose females at the interim necropsy (values were similar to controls at study termination). Non-neoplastic changes at histopathology included increased hepatocellular hypertrophy and fat storage (fatty change, intracytoplasmic vacuoles)(interim necropsy in 2500 ppm males; final necropsy in 1250 ppm males and in both sexes at 2500 ppm), hepatocellular necrosis (final necropsy in both sexes at 2500 ppm), and hepatocellular degeneration (final necropsy in males at 1250 and 2500 ppm). Adrenal effects were noted for females at 1250 and 2500 ppm. Hypertrophy of the cortical x-zone of the adrenals (2500 ppm interim necropsy) and vacuolization (intercurrent deaths and final necropsy at 1250 (48/50 versus 33/49 in control) and 2500 ppm (50/50)) were recorded. An increased number of eosinophilic, luteinized cells in the ovarian stroma or in surrounding adipose tissue were seen in females at 1250 and 2500 ppm at terminal sacrifice (ns). These effects along with the adrenal changes were attributed to hormone imbalance induced by liver enzyme induction (aromatase activity). In kidney, at the interim sacrifice, a decrease of sex-specific renal vacuoles was seen in all treated males. Also, lipid vacuolization in the medullary regions of the mandibular and mesenteric lymph nodes was noted at final necropsy for both sexes at 1250 and 2500 ppm. Neoplastic lesions noted included an increase in the incidence of ovarian luteomas in female mice with increasing dose. The incidence was 0/1/5/6 at 0, 30, 1250, and 2500 ppm respectively (this was also interpreted as secondary to liver enzyme induction (aromatase activity) by YRC 2894 resulting in a secondary hormone imbalance). All other neoplastic lesions were comparable to the control incidence and no differences were recorded in number of animals with tumors, number of benign or malignant tumors, or in time to tumor relative to controls. Chronic NOEL = 30 ppm (increased hepatocellular hypertrophy, necrosis, and degeneration, increased leukocyte counts at 1250 and 2500 ppm). Possible adverse effect: a dose-related increase in ovarian luteomas. Acceptable. (Green and Gee, 8/25/04).

**\*\*52966-0084, 0085 209552, 209553**, "YRC 2894, Subchronic Range-Finding Study for a Two-

Year Study in B6C3F1 Mice (Administration in Feed Over About 14 Weeks)", (U. Wirtzinger and C. Rühl-Fehlert, Bayer AG, Fachbereich Toxikologie, Wuppertal, Germany, Bayer Report No. 106868, 14 March 1995). 10 B6C3F1 mice per sex per group received YRC 2894 (98.6% thiacloprid) in the diet (containing 1% peanut oil) at 0 (basal diet), 50, 250, 1250, and 6250 ppm for 14 weeks. The mean daily intake of YRC 2894 during the treatment period was 19.9, 102.6, 542.4, and 2819.9 mg/kg/day for males and 27.2, 139.1, 704.3, and 3351.0 mg/kg/day for females at 50, 250, 1250, and 6250 ppm respectively. Group mean male bodyweights were reduced (14%) at 6250 ppm. Female bodyweights were comparable to controls at all treatment levels. Water consumption was reduced in males ( $p \leq 0.01$ ) and females ( $p \leq 0.05$ ) at the high dose level. Group mean relative liver weights were increased at 1250 (M: 9%, F: 10%) and 6250 ppm (M: 39%, F: 42%) for both sexes. No ophthalmology was conducted. Hepatocellular hypertrophy was increased at 250 ppm for males (0 at 0 ppm vs 6 at 250 ppm) and at 1250 (9/M, 10/F) and 6250 ppm (10/M & F) for both sexes. All high dose males showed severity grade 4 (marked); females grades ranged from slight to moderate with no degenerative changes. Increased N-demethylase activity was noted in liver tissue for males at 250 ppm and for both sexes at 1250 and 6250 ppm. P450 content was also increased for both sexes at 1250 and 6250 ppm. There were no toxicologically-relevant changes in hematology or clinical chemistry with dose. A reduction/loss of sex specific vacuolation in the proximal tubules of kidneys was recorded at 1250 and 6250 ppm in males. Record 209553 (Bayer 106868-1) contains additional histopathology of the adrenal glands, female genital tract and mammary glands and an immunohistochemical evaluation of prolactin on the pituitary gland of females at 6250 ppm. In females at 50 ppm and higher, a dose-related increase in the severity/grade of vacuolation of the x-zone of the adrenal cortex leading to hypertrophy was noted. Incidence in the control animals was high (9 at 0 ppm vs 10 at 50 ppm and higher), only the severity/grade increased with dose. In control animals, minimal to mild vacuolation was noted. At 50 ppm and higher vacuolation was increased leading to hypertrophy and enlargement of the x-zone. At 1250 ppm and higher the change was rated as marked or massive. In the ovaries, a reduced number of advanced corpora lutea with eosinophilic cells were recorded at 1250 ppm and higher. No evidence of changes in oviducts, uterus, vagina, mammary gland, or male adrenal glands and no changes were induced in the prolactin-secreting cells in the anterior pituitary. NOEL = 50 ppm (liver changes in males). The significance of the adrenal cortical and ovary changes in females remains unclear since both the adrenals and ovaries depend on complex endocrine regulation mechanisms that were beyond the scope of the study. Acceptable. (Green and Gee, 8/23/04).

## REPRODUCTION, RAT

**\*\*52966-055 209519**, "A Two-Generation Dietary Reproduction Study in Rats Using Technical YRC 2894", (D.A. Eigenberg, Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS., Study No. 95-672-W, Report No. 107628, 8 December 1997). 30 Sprague-Dawley rats per sex per group received YRC 2894 (96.7% thiacloprid) in the diet at 0, 50, 300, and 600 ppm through 2 generations (one mating per generation) beginning 10 weeks prior to cohabitation of the F<sub>0</sub> parents. Average mg/kg/day intakes of YRC 2894 (average of mean doses of both F<sub>0</sub> and F<sub>1</sub> animals) were 3.5 (M) and 3.7-6.8 (F), 21 (M) and 22-42 (F), and 41 (M) and 43-86 (F) mg/kg/day at 50, 300, and 600 ppm respectively. Lower bodyweights were noted for F<sub>0</sub> females and F<sub>1</sub> males and females at 600 ppm during the premating phase and for females during gestation and lactation. Increased food consumption was recorded for F<sub>1</sub> males and females at 600 ppm during the premating phase. Relative liver weights were increased (statistically significant) for F<sub>0</sub> and F<sub>1</sub> males and females at 600 ppm and for F<sub>0</sub> males and F<sub>1</sub> females at 300 ppm. Relative thyroid weights were increased (statistically significant) at 600 ppm for F<sub>0</sub> and F<sub>1</sub> animals of both sexes and for F<sub>0</sub> females and F<sub>1</sub> males at 300 ppm. Histopathology revealed hepatocytomegaly (minimal to slight in severity) in F<sub>0</sub> and F<sub>1</sub> animals of both sexes at 300 and 600 ppm. Minimal to moderate hepatocellular necrosis occurred in each of the mid and high dose group F<sub>0</sub> animals which died or were sacrificed due to dystocia. Thyroid follicular cell hypertrophy was increased in high dose F<sub>0</sub> males and in mid and high dose F<sub>0</sub> females and F<sub>1</sub> animals of both sexes. Parental NOEL = 50 ppm based on histopathology of liver and thyroid. Reproductive NOEL = 50 ppm based on dystocia in F<sub>0</sub> females at 300 and 600 ppm. Offspring NOEL = 50 ppm (reduced

pup weight). **Possible adverse effect:** dystocia. Acceptable. (Green and Gee, 8/27/04).

52966-0060 209524, "A Two-Generation Reproduction Range-Finding Study with YRC 2894 Technical in Rats", (M.C. Porter, V. Jasty, D.S. Grosso, and R.E. Hartnagel Jr., Toxicology - Healthcare, Miles, Inc. (Name changed to Bayer Corporation 1 April 1995), Elkhart, IN., Bayer Report No. 107043, 2 June 1995). 7 Crl:CD BR rats per sex per group received YRC 2894 (98.6% thiacloprid) at 0, 100, 400, and 1600 ppm through 1 generation (1 litter to 5 weeks postpartum) beginning 4 weeks prior to cohabitation. Food consumption and bodyweight were reduced ( $p \leq 0.01$ ) for F0 animals at 1600 ppm. Microscopy revealed increased hepatocellular hypertrophy in all F0 and F1 animals at 1600 ppm and in 1 mid dose (F0) female. Additionally, increases in thyroid follicular cell hypertrophy were noted for F0 animals at the high dose and in one male at the mid dose. Slight increases were also noted for F1a animals at the high dose level. F1a pup deaths were increased during post-partum days 0-4 at 1600 ppm. Pup growth was also reduced at the high dose: 21 day pup weights were significantly lower ( $p \leq 0.01$ ). Parental NOEL = 100 ppm (liver (hepatocellular hypertrophy) and thyroid (follicular cell hypertrophy) changes at 400 and 1600 ppm). Reproductive NOEL = 1600 ppm. Pup NOEL = 400 ppm (bodyweight, early deaths). Supplemental data. (Green and Gee, 8/30/04).

52966-0047 209511, "A reproduction Study in Rats to Determine If Administration of Technical YRC 2894 From Gestation Days 18 to 21 Will Cause Dystocia", (D.A. Eigenberg, Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Bayer Report No. 107639, 24 July 1998). 36 presumed pregnant CD Sprague-Dawley female rats received YRC 2894 (96.7% thiacloprid) by gavage at 100 mg/kg/day on gestation days 18 and 19, and 50 mg/kg/day on day 20 (due to toxicity at 100 mg/kg/day). 10 presumed pregnant control animals were also on the study. The study also evaluated an alternate light cycle on synchronizing parturition (lights were on at night instead of during the day and the light cycle was 14 hours instead of 12). Half of the animals were on the altered light cycle, the rest on the normal one. Toxicity was apparent in treated animals. Reduced bodyweight, increased mortality, and hypoactivity and reduced and/or no stool were noted. 7 control and 26 treated animals were pregnant and all control and 21 treated animals had normal deliveries. 4 treated dams died or were sacrificed during delivery. One of them was classified as having dystocia but toxicity precludes evaluation as a compound induced effect. The alternate light cycle did not synchronize/alter parturition. The results of this study did not alter the evaluation results for record 209519. (Green and Gee, 8/30/04).

52966-0053 209517, "Further Examination of the Increased Occurrence of Dystocia and Stillbirths Observed in a Reproductive Bioassay with an Experimental Cyanamide (YRC 2894)", (W.R. Christenson, Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Bayer Report No. 108360, 31 August 1998). Sprague-Dawley male and female rats (6 to 8 weeks of age at the start of treatment) were exposed to YRC 2894 (97%) in the diet for approximately 10 weeks for males (pre-mating phase) and up to 14 weeks for females (premating, gestation, and lactation) at 0 (basal diet) and 800 ppm (54.0 and 61.0 mg/kg/day for males and females respectively). 11 to 16 females per group were sacrificed for evaluation at four time points: (1) during the premating period (treatment week  $9 \pm 1$ ); (2) at gestation days 18; (3) or 21; (4) and on lactation day 2. Circulating levels of hormones (estrogen/estradiol, thyroxine ( $T_4$ ), triiodothyronine ( $T_3$ ), thyrotropin (TSH), follicle stimulating hormone (FSH), lutenizing hormone (LH), progesterone, corticosterone, prolactin, oxytocin), uterine and cervical levels of glutathione and prostaglandin, and uterine estrogen and progesterone receptor concentrations were measured. Bodyweight gain was slightly reduced for 800 ppm dams during premating and gestation with no change in food consumption. Relative liver weights were increased ( $p \leq 0.05$ ) for 800 ppm dams at all time points and histopathology revealed centrilobular hepatocytomegaly and proliferation of smooth endoplasmic reticulum (SER). No histological changes were seen in the hypothalamus, pituitary, ovary or adrenal gland. Cytochrome P450 activity (N-demethylase and O-demethylase) was also elevated ( $p \leq 0.05$ ) at all time points. Two 800 ppm dams had difficulty delivering (dystocia). One delivered several pups on gestation day 23 and was necropsied 24 hours later with 4 pups *in utero* (2 live, 2 dead). The other female had a bloody vaginal discharge on gestation day 22 but delivered no pups. At necropsy, on day 23, one pup was found in the birth

canal with additional dead pups *in utero*. Circulating levels of estrogen/estradiol, progesterone, corticosterone, and lutenizing hormone were elevated ( $p \leq 0.05$ ) at all time points for 800 ppm females. Estrous cyclicity was monitored. Cervical and uterine prostaglandin and GSH levels were generally similar to controls. Nuclear and cytosolic estrogen and progesterone receptor concentrations were not affected by treatment. Data suggest changes in circulating steroid and steroid-related hormone levels may be a secondary response to a liver effect from YRC 2894 treatment. Supplemental data. (Green and Gee, 9/1/04).

52966-0054 209518, "An Experimental Study to Investigate the Cause of Dystocia and Stillbirths in Rats Treated with Technical Grade YRC 2894", (D.A. Eigenberg, Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Bayer Report No. 107766, 2 September 1998). 30 male and 155 female CD Sprague-Dawley rats (7 weeks of age) per group received YRC 2894 (97.0% thiacloprid) in the diet at 0 (Purina Rodent Laboratory Chow 5001-4: etts form) and 1000 ppm (937 ppm analyzed) beginning 10 weeks prior to mating through parturition. Males were sacrificed and discarded after mating. Intake was equivalent to 62 and 73 mg/kg/day for males and females, respectively, during premating. 4 females were found dead (one each on gestation days 15, 18, and 22, and one (not pregnant) on study day 92) at 1000 ppm. Statistically significant lower female bodyweights (2% to 6% below control values) were recorded from day 14 of the premating phase at 1000 ppm. The mean number of fetuses per litter was reduced ( $p \leq 0.05$ ) at 1000 ppm (12.3 fetuses/34 litters at 0, vs 10.2 fetuses/34 litters at 1000 ppm). No gross or histopathologic changes were noted in the cervix and uteri. The possible mechanistic causes of dystocia were evaluated. Light Induced Fluorescence (a measure of cervical collagen concentration). Measurements were conducted in the laboratory of Dr. Garfield at the University of Texas Medical Branch (UTMB) by Wenling Sha Glassman and Shao-Qing Shi. Rats were anesthetized and the fluorescence spectra was obtained from the external os of the cervix using a probe that contained an optical fiber bundle in the center. Rats were evaluated every other day from gestation day 13 and on day 22 (day of delivery). Results showed significant differences ( $p \leq 0.05$ ) between control and treated rats on gestation days 13, 15, and 17, and no statistical difference on days 19, 21, and 22. Thus, the cervix were harder in YRC 2894 treated rats than in control animals at mid pregnancy. Based on the absence of an effect on the cervix prior to or at parturition, the dystocia observed in record 209519 was not attributed to a YRC 2894 effect on cervical collagen. Cervical Extensibility and cervical wet and dry weight were determined by O. David Sherwood at the University of Illinois (10 dams per group on gestation day 21) and by UTMB (Dr. Garfield) (6 dams per group were evaluated on gestation days 16 and 21; and 4 control and 2 treated animals on gestation day 22). Cervical tensile properties were measured by suspending the cervix between 2 hooks, one hook was stationary and the other was attached to a force displacement transducer. The slopes for the grams of tension/mm extension were determined for each cervix. No differences in cervical wet weight, dry weight, or water content between the groups were observed and no differences were found between the groups for the mean slopes of grams tension/mm extension. Uterine Contractility was assayed for 4 to 6 control and treated dams on gestation days 16 and 22. The effect of oxytocin solutions ( $10^{-10}$  to  $10^{-6}$  M) and isoproterenol solutions ( $10^{-11}$  to  $10^{-6}$  M) (added in 10  $\mu$ l to 23  $\mu$ l increments) on the contraction of isolated rings of uterus was evaluated. No difference between control and treated tissue was found. EMG Recordings (measure of electrical activity of the uterus) and Intrauterine Pressure were measured daily (for 4 hours) in 5 control and 3 YRC 2894 treated rats from gestation day 18 through termination of delivery. For the EMG recordings, rats were anesthetized on gestation day 15, the abdominal cavity was opened, and the electrodes (a pickup electrode and a ground) of the telemetric device were sutured to the uterine wall (approximately mid distance between the ovarian and cervical ends of the uterine horn). For intrauterine pressure measurement, catheters were introduced through the uterine wall into the uterine cavity in the same approximate location as the uterine electrodes. No significant difference between control and treated rats for intrauterine pressure or electrical activity was found. Quantification of Uterine Alpha-1 Adrenergic Receptors was performed on uteri from 15 pregnant control and treated rats sacrificed on gestation day 21. Fetuses and placentas were removed and the uterus and cervix were homogenized and centrifuged (1000 x gravity for 10 minutes). Pellets were discarded and the remaining supernatants centrifuged (49000 x gravity for 20 minutes). Supernatants were

discarded and the pellets (containing the plasma membranes) were resuspended in glycylglycine buffer and frozen in liquid nitrogen. After thawing, binding assays were conducted (in duplicate) using 130 microliters of the plasma membrane preparation from each tissue which was incubated (room temperature for 30 minutes) with radiolabelled receptor ligand ( $[^3\text{H}]$ -Prazosin) in the presence or absence of unlabelled ligand (Norepinephrine). After incubation, preparations were filtered through Millipore Type APFC filter paper. Each filter was washed (glycylglycine buffer) and the radioactivity counted. The number of Alpha-1 receptors was quantified by subtracting the nonspecific binding results (radiolabelled prazosin and unlabelled norepinephrine) from total binding results (radiolabelled prazosin and buffer). Mean alpha-1 receptor levels for control and treated dams were similar (3.85 and 3.89 pmol/receptor/mg protein, respectively) and not effected by treatment. Pathology Report. Ten females from each group were given a histological evaluation of the cervix and uterus on gestation day 21, including organ weights. Mean bodyweight at 1000 ppm was statistically lower (327.1 g\* versus 384.4g) and relative ovarian weight increased. No micropathology findings were considered due to treatment. No change to record 209519. (Green and Gee, 8/31/04).

52966-0056 209520, "A One-Generation Dietary Reproduction Study in Rats Using Technical Grade YRC 2894 to Evaluate the Reproducibility of Dystocia and an Increase in Stillbirths in the P Generation of a Two-Generation Dietary Reproduction Study in Rats", (D.A. Eigenberg, Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS., Bayer Report No. 107641, 12 May 1998). 15 male and 30 female Sprague-Dawley rats per group received YRC 2894 Technical (97.0% thiacloprid) in the diet at 0 (basal diet), 25, 300, and 1000 ppm through 1 generation (1 litter). Treatment began 10 weeks prior to mating. Pups were sacrificed and discarded on lactation day 4. Group mean analyzed intakes of YRC 2894 were 2 (M and F), 20 (M) and 20 to 35 (F), and 69 (M) and 68 to 119 (F) mg/kg/day at 25, 300, and 1000 ppm respectively. The incidence of paleness, labored breathing, and cold to touch was increased for 1000 ppm females. 4 high dose females died during gestation days 22 to 24, one died on study day 40 (premating), and 1 was sacrificed moribund on study day 134. Two of the dams died during difficult deliveries (some pups were delivered followed by a long period of time with no further deliveries and with pups found *in utero* at death) and were considered dystocic. Bodyweights were reduced for high dose females during premating, gestation, and lactation. No change in food consumption. Increased mean relative liver and thyroid weights were recorded for F0 females at 1000 ppm and at 300 ppm for liver. Pup viability was decreased at 1000 ppm. Mean live litter size was reduced at birth (12.5 at 0 vs 9.9 at 1000 ppm) and on lactation day 4 (11.7 at 0 vs 8.4 at 1000 ppm). Day 4 pup weights were also reduced ( $p \leq 0.05$ ) (10.3 g at 0 vs 8.9 g at 1000 ppm). Parental NOEL = 25 ppm (increased liver weight at 300 ppm and lower bodyweight, labored breathing, death at 1000 ppm). Reproductive NOEL = 300 ppm (dystocia and decreased pup viability at 1000 ppm). Pup NOEL = 300 ppm (body weight). No adverse effect (maternal toxicity precludes a mechanistic determination for dystocia). Supplemental information. (Green and Gee, 8/30/04).

52966-0057 209521, "A reproduction Study in Rats to Determine If Administration of Technical YRC 2894 From Gestation Days 18 to 21 Will Cause Dystocia (Study Number II)", (D.A. Eigenberg, Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Bayer Report No. 107640, 4 May 1998). 30 mated CD Sprague-Dawley female rats per group received YRC 2894 (97.0% thiacloprid) by gavage at 0 (aqueous 0.5% (w/v) carboxymethylcellulose with 0.4% (v/v) Tween 80), 35 and 60 mg/kg/day on gestation days 18 through 21. Because of maternal toxicity and death at 35 and 60 mg/kg/day, an additional group was added at 17.5 mg/kg/day. Females (total of 9) from the other groups that had not reached gestation day 18 (and, therefore, remained untreated) were used. Hypoactivity, chromorhinorrhea, and clear vaginal discharge were recorded for females at 35 and 60 mg/kg/day. 5 females died at 35 mg/kg/day, 7 at 60 mg/kg/day. 2 and 1 dams were sacrificed moribund at 35 and 60 mg/kg/day respectively. No deaths at 17.5 mg/kg/day. Statistically significant lower bodyweights were recorded for 35 and 60 mg/kg/day females (5% and 3% lower than controls respectively on day 19 and 14% lower (both groups) on gestation day 21). Bodyweight gain was decreased (statistically significant from control (41.3 g.)) at 17.5 (13.3 g.), 35 (-18.3 g.), and 60 mg/kg/day (-24.9 g.) for gestation days 18-21. Statistically significant reductions in food consumption of 35% to 54% at 17.5 mg/kg/day



and 81% to 94% at 35 and 60 mg/kg/day compared to controls were recorded after the first dose. The live birth index was decreased ( $p \leq 0.05$ ) at 35 and 60 mg/kg/day compared to controls. The number of stillborn pups were 2, 5, 28, and 34 at 0, 17.5, 35, and 60 mg/kg/day respectively. The marked maternal toxicity precludes characterization of the increase in stillborns as compound induced. No dystocia. No change to record 209519. (Green and Gee, 8/30/04).

52966-0070 209534, "YRC 2894: Determination of Aromatase Activity in Ovary and Liver Tissue of a Modified 1-Generation Reproductive Study in Sprague-Dawley Rats", (U. Schmidt, Bayer Corporation, Department of Toxicology, Wuppertal, Germany, Study No. T6 062 080, Bayer Report No. 108513, 27 July 1998). In record 209517, Sprague-Dawley male and female rats (6 to 8 weeks of age at the start of treatment) were exposed to YRC 2894 in the diet for approximately 10 weeks for males (pre-mating phase) and up to 14 weeks for females (premating, gestation, and lactation) at 0 (basal diet) and 800 ppm (54.0 and 61.0 mg/kg/day for males and females respectively). Ovary aromatase activity (cytochrome P450 19) was measured in tissue taken from 6 females per group during premating (treatment week  $9 \pm 1$ ), on gestation day 18, and on lactation day 2 and correlated with the circulating estrogen/estradiol concentration. Group mean aromatase activity in 800 ppm ovaries was similar to that of control tissue during premating (3.7 pmol/g/min. at 0 vs 3.6 pmol/g/min at 800 ppm) and on gestation day 18 (23.1 pmol/g/min at 0 vs 25.0 pmol/g/min at 800 ppm), and increased ( $p \leq 0.05$ ) ( $10 \pm 4.5$  pmol/g/min. at 0 vs  $26.1 \pm 12.4$  pmol/g/min at 800 ppm) on lactation day 2. Note: 2/6 dams did not deliver litters. However, serum estrogen/estradiol levels were elevated at all three time points for 800 ppm animals, suggesting aromatase activity of the ovaries was not directly affected by treatment. Additionally, induction of cytochrome P450 dependent aromatase activity in liver was evaluated in microsomes from the same rats at the premating time point and was found to be significantly increased ( $p \leq 0.01$ ) (7.5 pmol/g/min at 0 vs 14.7 pmol/g/min at 800 ppm) at 800 ppm. This may indicate the increased serum estrogen/estradiol concentration was partly a result of increased synthesis through treatment-related induction of aromatase cytochrome P450 in the endoplasmic reticulum of liver. Supplemental. (Green and Gee, 8/25/04).

52966-0062 209526, "YRC 2894: Special Study for Subacute Oral Toxicity in Rats (Toxicokinetics in Pregnant and Non-Pregnant Rats)", (P. Andrews and U. Schmidt, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Study Number T3061538, Bayer Report No. 108511, 14 July 1998). This study investigated the effect of gestation on the pharmacokinetic behavior of YRC 2894 (97.2%) in blood plasma of female Sprague Dawley rats. 7 male and 8 female rats received YRC 2894 in the diet at 1000 ppm from mating through gestation (males were discarded after mating). Another group of 12 non-pregnant females received the same treatment over the same time period. 5 pregnant and 5 non-pregnant females served as controls. A difference between YRC 2894 plasma levels in pregnant and non-pregnant treated females per time was indicated. In non-pregnant females, mean concentrations were 63.2, 61.8, 58.8, and 56.6 nmo/l at day 0, 7, 14, and 21 respectively. In pregnant animals, significant increases were noted for days 7 (83.6 nmol/l,  $p \leq 0.01$ ), 14 (71.1 nmol/l,  $p \leq 0.05$ ), and 21 (85.7 nmol/l,  $p \leq 0.01$ ) compared to day 0 (57.3 nmol/l) levels. The authors conclude that toxicity as a result of increased systemic exposure in pregnant animals may have been related to the dystocia observed in record 209519. Supplemental. (Green and Gee, 8/30/04).

#### TERATOLOGY, RAT

\*\* 0174; 216850; "YRC 2894, Developmental Toxicity Study in Rats After Oral Administration"; (B. Stahl; Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany; Report No. 108869; 2/13/97); Thirty five mated female rats/group were treated orally by gavage with 0 (aqueous 0.5% carboxymethyl cellulose), 2, 10, or 50 mg/kg/day of YRC 2894 (batch no. 290894, purity: 97.0 to 97.3%) from day 6 through day 19 of gestation. The mean body weight gain and food consumption of the 50 mg/kg group dams were less than those values for the control ( $p < 0.01$ )

with actual weight loss for the 50 mg/kg dams between days 6 and 9 of gestation. There was an increased number of late resorptions ( $p < 0.01$ ), lower mean fetal weight ( $p < 0.01$ ) and an increased incidence of limb bone dysplasia (litter, 0: 1/28 vs. 50: 6/29,  $p = 0.056$  (Fisher exact)) for the 50 mg/kg treatment group. **No adverse effect indicated** (no treatment-related fetal effects were evident at the Maternal NOEL); **Maternal NOEL:** 10 mg/kg/day (based upon the reduced body weight gain and food consumption of the 50 mg/kg treatment group); **Developmental NOEL:** 10 mg/kg/day (based upon the increased numbers of late resorptions, lower mean fetal weight, and increased incidence of limb bone dysplasia noted for the 50 mg/kg treatment group); **Study acceptable.** (Moore, 5/18/05)

#### TERATOLOGY, RABBIT

**\*\*52966-0086** 209554, "YRC 2894, Developmental Toxicity Study in Rabbits After Oral Administration", (B. Holzum, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. 24709, Bayer 108868, 9 January 1996). 24 mated female Himalayan CHBB:HM rabbits per group received YRC 2894 (97.3% thiacloprid) by oral gavage at 0 (0.5% carboxymethylcellulose in demineralized water), 2, 10, or 45 mg/kg/day on gestation days 6 through 28. Food consumption was decreased ( $p \leq 0.01$ ) at 10 and 45 mg/kg/day. Bodyweight gain was reduced ( $p \leq 0.01$ ) at 10 and 45 mg/kg/day. An increase in the number of does with decreased water intake was noted at 45 mg/kg/day. One female receiving 2 mg/kg/day aborted on gestation day 24. At 45 mg/kg/day, 2 females aborted (one on gestation day 23, the other on day 28) and 3 had total litter resorptions. Fetal weight was marginally decreased at 10 mg/kg/day and significantly ( $p \leq 0.01$ ) so at 45 mg/kg/day. Retarded fetal skeletal ossification was also noted at 45 mg/kg/day. Common fetal skeletal malformations were marginally increased at 45 mg/kg/day (arthrogryposis; 3 at 0 (2 litters) vs 5 at 45 mg/kg/day (2 litters) and supernumerary lumbar vertebra with supernumerary 13<sup>th</sup> ribs; 0 at 0 vs 2 at 45 mg/kg/day (2 litters)). Maternal NOEL = 2 mg/kg/day (reduced food consumption and bodyweight gain at the mid and high dose). Developmental NOEL = 2 mg/kg/day (reduced fetal weights at 10 and 45 mg/kg/day). No adverse effects. No teratogenicity. Acceptable. (Green and Gee, 8/27/04).

#### GENE MUTATION

**\*\*52966-0048** 209512, "YRC 2894, Reverse Mutation Assay (*Salmonella typhimurium* and *Escherichia coli*)", (Kaoru Ohta, Nihon Bayer Agrochem K.K., Research & Development Division, Hino Institute, Tokyo, Japan, Report No. RA95011, Bayer 107410, 21 August 1995). Cultures (glucose agar plates) of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA were exposed to YRC 2894 (96.8% thiacloprid) at 0 (DMSO), 313, 625, 1250, 2500, and 5000  $\mu\text{g}/\text{plate}$  for 48 hours in the presence and absence of S9 in two trials. Strains were preincubated for 20 minutes with YRC 2894 solutions in test tubes, in the presence and absence of S9, prior to plating. Crystallization was noted at 5000  $\mu\text{g}/\text{plate}$ . No increase in the number of revertants. Acceptable. (Green and Gee, 8/23/04).

**\*\*52966-0074** 209538, "YRC 2894, Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HPRT Assay *In Vitro*", (S. Brendler-Schwaab, Bayer AG, Fachbereich Toxicology, Wuppertal, Germany, Report No. 25163, 13 June 1996). Exponentially growing V79 cells ( $4 \times 10^6$ ) were plated (250 ml flasks) in duplicate and treated with YRC 2894 (96.8% thiacloprid), in the presence and absence of rat liver S9, at untreated, 0 (DMSO), 15.6, 31.3, 62.5, 125, 250, and 500  $\mu\text{g}/\text{ml}$  for 5 hours. 2 trials without and 3 trials with S9 were performed. No increase in forward mutations was indicated. Positive controls were functional. Acceptable. (Green and Gee, 8/23/04).

#### CHROMOSOME EFFECTS

**\*\*52966-0046** 209510, "YRC 2894, *In Vitro* Mammalian Chromosome Aberration Test with Chinese Hamster V79 Cells", (B. Herbold, Bayer AG, Fachbereich Toxicology, Wuppertal, Germany, Report No. 24516, Bayer Report No. 107180, 24 November 1995).  $1 \times 10^6$  Chinese

hamster V79 cells were seeded in duplicate in 20 ml of medium per 75 cm<sup>2</sup> flask and exposed to YRC 2894 (96.8% thiacloprid) in the presence and absence of S9 at 0 (DMSO), 0 (untreated), 75, 300, and 750 µg/ml for 4 hours. Cells were harvested and evaluated 18 hours after the start of treatment and additionally at 30 hours for the 750 µg/ml cultures. 100 metaphases per culture were evaluated by light microscopy. Cytotoxic effects (decreased mitotic index) were noted at 750 µg/ml in the presence and absence of S9 at 18 hours. Aberrant metaphases were not increased. Acceptable. (Green and Gee, 8/23/04).

\*\*52966-058 209522, "YRC 2894, Micronucleus Test on the Mouse", (B. Herbold, Bayer Ag, Fachbereich Toxicology, Wuppertal, Report No. 24515, Bayer Report No. 107179, 24 November 1995). 5 Hsd/Win: NMRI mice per sex per group received YRC 2894 (96.8% thiacloprid) once by intraperitoneal injection at 0 (0.5% aqueous Cremophor) and 60 mg/kg followed by bone marrow sampling 16, 24, and 48 hours later. 3 animals died after treatment. Apathy, roughened fur, spasm, and difficulty breathing were recorded for up to 16 hours after treatment. Slides of the bone marrow were evaluated by light microscope. 1000 polychromatic erythrocytes were examined per animal. There was no increase in micronucleated polychromatic erythrocytes. The positive control was functional. Acceptable. (Green and Gee, 8/23/04).

### DNA DAMAGE

\*\*52966-0073 209537, "YRC 2894, Test on Unscheduled DNA Synthesis in Rat Liver Primary Cell Cultures *In Vitro*", (S. Brendler-Schwaab, Bayer AG, Fachbereich Toxicology, Wuppertal, Germany, Report No. 25429, 16 September 1996). Hepatocyte cultures (3.75 x 10<sup>5</sup>) were treated in triplicate with YRC 2894 (97.2% thiacloprid) in the presence of <sup>3</sup>H-thymidine at 0 (DMSO), 75, 150, 300, 350, 400, 450, and 500 µg/ml for 16-24 hours. 50 cells per slide were counted. Net grains per nucleus and the number of cells in repair were not increased by treatment. Positive controls were functional. Acceptable. (Green and Gee, 8/23/04).

### SUPPLEMENTAL STUDIES

52966-0064 209528, "Mechanistic Studies on Aromatase Induction and Toxicokinetics in Rats (4-Week Feeding Studies)", (P. Andrews, W. Bomann, F. Krotlinger, and U. Schmidt, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Study Numbers T 0061940 and T 3062311, Bayer Report No. 108516, 27 July 1998). Two studies were performed to investigate the effect of dietary administration of YRC 2894 on aromatase activity in male and female rats. In the first study, 15 Wistar (Hsd Cpb: WU) rats per sex per group received YRC 2894 (96.2% thiacloprid) in the diet at 0 (basal diet + 1% peanut oil), 100, and 1000 ppm for 4 weeks. In the second study, groups of 10 female Wistar rats received YRC 2894 in the diet at 0, 200, and 500 ppm for 4 weeks. The mean intake of YRC 2894 was 6.7 and 66.7 mg/kg/day for males and 6.6 and 60.4 mg/kg/day for females at 100 and 1000 ppm respectively and 20.4 and 47.5 mg/kg/day for females at 200 and 500 ppm respectively. There were no deaths and no treatment related clinical signs. Group mean bodyweights were reduced (p≤0.01) at 1000 ppm for males (8%) and females (6%) in the first study, and, at 500 ppm for females (9%) in the second study. Food consumption was slightly reduced (NS) for males at 100 ppm and for both sexes at 1000 ppm (first study) and for females at 200 and 500 ppm (second study). Group mean relative (p≤0.01) and absolute liver weights were increased for both sexes at 1000 ppm (first study) and for 500 ppm females (second study). Group mean aromatase (cytochrome P450 19) activity in liver microsomes was significantly increased for females at 1000 ppm (p≤0.05, 10.4 pmol/g/min at 0 vs 23.0 pmol/g/min at 1000 ppm) in the first study and at 200 (p≤0.01, 9.1 pmol/g/min at 0 vs 16.4 pmol/g/min at 200 ppm) and 500 ppm (p≤0.05, 19.2 pmol/g/min) in the second study. Ovary aromatase activity was not altered by treatment through 1000 ppm in the first study (4.2 pmol/g/min at 0 vs 4.3 pmol/g/min at 100 ppm and 4.4 pmol/g/min at 1000 ppm). YRC 2894 plasma concentrations were fairly constant and dose-proportional in treated males through day 28. Group mean plasma concentrations in males were 6.7, 7.1, 7.3, 7.3, and 5.8 nmol/ml at 100 ppm and 59.9, 64.3, 52.1, 48.7, and 52.3 nmol/ml at 1000 ppm on study days 1, 8, 15, 22, and 28

respectively. In females, group mean plasma concentrations were elevated at 1000 ppm on days 8, 15, 22, and 28 (96.8, 89.6, 80.4, and 84.0 nmol/ml respectively) compared to the day 1 value (51.6 nmol/ml). Plasma levels in females treated at 100 ppm were generally similar to male values at the various time points. NOEL for aromatase induction in liver was 100 ppm. Supplemental. (Green and Gee, 8/25/04).

52966-0065 209529, "YRC 2894: Mechanistic Studies on Aromatase Induction in Mice (Feeding Study for 13 Weeks)", (P. Andrews, W. Bomann, C. Ruhl-Fehlert, and U. Schmidt, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Study Number T 7061541, Bayer Report No. 108519, 27 July 1998). This study was performed to determine the effect of dietary administration of YRC 2894 on aromatase activity in hepatic tissue and potential hormonal changes in female B6C3F1 mice. 30 females per group received YRC 2894 (97% thiacloprid) in the diet at 0 (basal diet), 0 (untreated control), 10, 30, 250, and 2500 ppm for up to 13 weeks (15 per group were necropsied after 4 weeks, remaining animals after 13 weeks (animals were in the diestrus phase at necropsy)). An additional group of 30 females also received 2500 ppm in the diet plus 0.005% mecamlamine (a nicotine mimicking agent) in the drinking water. Mean YRC 2894 intake measured over 28 days was 6, 18, 139, 1101, and 1244 g/kg of bodyweight/day at 10, 30, 250, 2500, and 2500 plus mecamlamine respectively. Decreased reactivity was observed at 250 (2/15 in the terminal necropsy group), and 2500 ppm (13/30. 3/15 were affected in 2500 ppm + mecamlamine (terminal necropsy group). Increased motility was observed at 250 (4/15) and 2500 ppm (2/15) from day 49. Decreased motility was observed in 2/15 animals at 2500 ppm from day 57. Food consumption was reduced at 2500 ppm and at 2500 ppm plus mecamlamine. Water consumption and bodyweight were not effected by treatment. Progesterone levels were increased at 2500 and 2500 ppm + mecamlamine (1.48 ng/ml at 0 vs 2.02 and 2.1 ng/ml respectively) after 13 weeks of treatment. The estradiol/progesterone ratio at 2500 ppm was 17.4 versus 26.1 for controls. Ratios at 250 ppm were similar to controls. Aromatase activity in hepatic tissue was increased ( $p \leq 0.01$ ) at 250 and 2500 ppm after 13 weeks of treatment (means were 11.9 pmol/g/min at 0 vs 19.6 and 56.2 pmol/g/min respectively). Mean absolute and relative liver weights were increased ( $p \leq 0.001$ ) at 2500 and 2500 ppm + mecamlamine at both the interim and terminal necropsies. A dose-related increase in vacuolation of the X-zone located in the inner adrenal cortex was found at 250, 2500, and 2500 ppm + mecamlamine at 4 and 13 weeks. Incidences at 4 weeks were: 0, 2/15; 250 ppm, 6/15; 2500 ppm, 11/15. At 13 weeks, the incidences were similar (12/15 at 0 and 15/15 at 250 and 2500 ppm) but the degree was increased from 1.0 for controls to 2.4 and 3.9 for 250 and 2500 ppm respectively. At 2500 ppm, the marked vacuolation resulted in an enlargement of the X-zone at 13 weeks. NOEL = 30 ppm (adrenal pathology, aromatase induction). Supplemental. (Green and Gee, 8/26/04).

52966-0069 209533, "YRC 2894, Investigation of the Inhibition of Cytochrome P450 Dependent Monooxygenases in Liver Microsomes (In Vitro)", (U. Schmidt, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Study Number T 6 053 684, Bayer Report No. 108514, 21 July 1998). This study investigated whether YRC 2894 inhibited cytochrome P450 dependent monooxygenases by measuring effects on 7-ethoxycoumarin-deethylation and testosterone hydroxylation *in vitro*. For the 7-ethoxycoumarin deethylase assay (ECOD), YRC 2894 was dissolved in DMSO (concentrations of 0, 0.1, 1, 10, and 100  $\mu$ M were used). 7-ethoxycoumarin, liver microsomes from rat and dog, Sorenson buffer,  $MgCl_2$  solution, albumin solution, and NADPH solution were added. Formation of 7-hydroxycoumarin was followed by fluorimetry ( $\lambda_{Excit}$  375 nm.  $\lambda_{Emis}$  453 nm). Fluorescence was plotted over a 5 minute period. Enzymatic activity was determined by comparison with the calibration standard. Determination of cytochrome P450 dependent metabolism of testosterone was performed at 37°C in 10 ml tubes (final volume of incubation mixture was 1.0 ml) containing 1mM NADPH, 2.5 mM  $MgCl_2$ , 40 mg/ml wet rat liver tissue in 0.1 M Sorensen buffer, and YRC 2894 (concentrations of 0, 10, 100, 500, and 1000  $\mu$ M were tested). Reaction was initiated by addition of testosterone in a methanolic stock solution (1mM). It was stopped after 15 minutes with addition of 5 ml dichloromethane. 100  $\mu$ l of corticosterone (100  $\mu$ M) was added. The resulting mixture was shaken then centrifuged for 10 minutes at 2000 x g. The residue was dried (under a stream of

nitrogen at 40°C), dissolved in 100  $\mu$ M methanol and analyzed using high performance liquid chromatography (HPLC). Inhibition was less than 50% at all concentrations of YRC 2894 for both species (i.e., 7-ethoxycoumarin deethylase inhibition ( $IC_{50} > 100 \mu$ M) in dog and rat liver microsomes and testosterone metabolism inhibition ( $IC_{50} > 1000 \mu$ M) in rat liver tissue). YRC 2894 was considered a “weak” inhibition of ECOD in microsomes as some inhibition occurred in 100  $\mu$ M. Supplemental. (Green and Gee, 8/25/04).

52966-0081 209549, “YRC 2894, Studies on the Inhibition of Thyroid Peroxidase-Catalyzed Reactions by YRC 2894 and Its Metabolites In Vitro (Revised Final from Report No. 23495)”, (Alexius Freyberger, Bayer AG, PH-PD Toxicology, Wuppertal, Germany, Report Number 23495 A, Bayer Report No. 108886, 28 January 1999). This study investigated the effects of YRC 2894 on thyroid peroxidase (TPO). It was performed *in vitro* using a partially purified hog thyroid fraction as an enzyme source. Plasma samples from YRC 2894 treated rats (2000 ppm in the diet for 14 days) were also screened for formation of TPO inhibitory metabolites. TPO-catalyzed Guaiacol oxidation. Guaiacol (5 mM), TPO (catalyzing 0.1  $\Delta E$  /min as measured by guaiacol oxidation) and YRC 2894 (870  $\mu$ M in 40  $\mu$ l DMSO) or solvent control were preincubated (room temperature, pH 7.4) for 1 minute then hydrogen peroxide (200  $\mu$ M) was added to initiate the reaction. The initial linear increase of absorption at 470 nm was used to calculate the peroxidase activity. TPO-catalyzed iodine formation. Incubations were performed as above with guaiacol replaced by potassium iodide (10 mM) and 150  $\mu$ M of hydrogen peroxide was added. The initial linear increase of the absorption at 350 nm was used to calculate enzyme activity. Detection of potential cyanamide formation during hydrolysis of YRC 2894. Suspensions of YRC 2894 (1.74 mM) in 0.2 M potassium phosphate buffer (pH 7.4) with 50  $\mu$ l DMSO or 10  $\mu$ l ethanol (to increase solubility) were incubated for up to 45 hours at 50°C. After cooling to room temperature, 0.5 ml samples were run in the TPO/guaiacol oxidation assay (870  $\mu$ M YRC 2894 and control) as described above. Formation of TPO inhibitory metabolites from YRC 2894 in vivo. Plasma samples (2.0 ml) from control and YRC 2894 treated rats (2000 ppm in the diet for 14 days) were extracted with 1.5 volumes ethylacetate. 80  $\mu$ l DMSO and 1.1 ml of 2.0 M potassium phosphate buffer (pH 7.4) containing 8% triton x-100 were added and the samples mixed and sonicated. 0.5 ml aliquots were then assayed in the TPO-catalyzed iodine system as described above. Neither YRC 2894 nor its metabolites had a direct, inhibitory effect on TPO-catalyzed reactions in the assays performed. Supplemental. (Green and Gee, 8/25/04).

## RAT METABOLISM

\*\*52966-0014 209478, “[Thiazolidine-4,5- $^{14}C$ ]YRC 2894: Absorption, Distribution, Excretion, and Metabolism in the Rat”, (H. Printz and W. Bornatsch, Bayer AG, Agrochemical Division, Plant Protection Development, Institute for Metabolism Research and Residue Analysis, Leverkusen-Bayerwerk, Germany, PF-Report No. 4299, Bayer Report No. 108290, 8 December 1997). 5 rats per sex per group or 5 male rats per group received a single oral gavage dose of [Thiazolidine-4,5- $^{14}C$ ] YRC 2894 at 1 or 100 mg/kg. Urine was collected separately for each animal in the group under cooling with dry ice at 0 to 4, 4 to 8, 8 to 24, and 24 to 48 hours post-dosing. Feces were collected at 0 to 24 and 24 to 48 hours after administration. Expired air was collected (1:1 solution of methanol/ethanolamine in wash bottles) separately for 5 males that received 1 mg/kg at 0 to 8, 8 to 24, and 24 to 48 hours after administration. Rats were sacrificed 48 hours after treatment. Radioactivity was quantified (liquid scintillation counting (LSC)) in erythrocytes, plasma, spleen, gastrointestinal tract (GIT), liver, kidneys, adrenal fat, adrenals, testes, ovaries, uterus, muscle, bone, heart, lung, brain, thyroid, skin, and carcass.

After treatment with 1 mg/kg between 72.7% (males) and 82.9% (females) of administered radioactivity was excreted in the urine during 48 hours. Between 10.47% (males) and 18.81% (females) was recovered in feces. After males received 100 mg/kg, 60.23% and 13.28% of the radioactivity was recovered in urine and feces respectively.

Maximum plasma levels of radioactivity were reached 2 (F) and 3 (M) (1 mg/kg), and 4 (100

mg/kg) hours post-dosing. Dose normalized concentrations (recovered per g plasma/administered per g bodyweight) were 0.709 and 0.775 for males and females respectively at 1 mg/kg and 0.544 at 100 mg/kg. 48 hours after dosing at 1 mg/kg, 0.031 and 0.018 group average % of given radioactivity had been recovered in plasma from males and females respectively at 1 mg/kg; the amount was 0.098 % in males at 100 mg/kg.

0.86% of administered radioactivity was found in expired air of males during the 48 hours following a 1 mg/kg oral gavage dose.

48 hours post-dosing, highest concentrations of radioactivity as % of dose were found in thyroid (3.14 for males and 3.48 for females) at 1 mg/kg. Thyroid values at 100 mg/kg were low in comparison (0.001). Measured concentrations of radioactivity on an organ/tissue to bodyweight basis (dose normalized concentration) were highest in liver (0.135 and 0.075 for males and females at 1 mg/kg and 0.374 at 100 mg/kg) and kidney (0.078 (males) and 0.048 (females) at 1 mg/kg and 0.202 at 100 mg/kg) followed by thyroid, adrenals, and spleen. Percent of dosed radioactivity concentrations were, for liver, 0.690 (males) and 0.299 (females) at 1 mg/kg and 1.934 at 100 mg/kg. Kidney values were 0.060 (males) and 0.035 (females) at 1 mg/kg and 0.148 at 100 mg/kg in comparison. The gastrointestinal tract in males that received 100 mg/kg showed levels of radioactivity (4.246%) twice as high as liver (1.934%). Gastrointestinal tract recoveries were generally comparable to liver at 1 mg/kg.

17 metabolites (including YRC 2894) were identified (high performance liquid chromatography (HPLC) and mass spectrometry) and quantified after isolation from pooled urine and 5 were identified in feces. All metabolites identified in feces were also found in urine. A proposed metabolic pathway of [Thiazolidine-4,5-<sup>14</sup>C] YRC 2894 started with hydroxylation of the carbon atom in position 4 of the thiazolidine ring (WAK 6856, 3-(6-chloro-pyridin-3-ylmethyl)-4-hydroxy-thiazolidine-2-ylidene-cyanamide) and its subsequent conjugation with glucuronic acid (PIZ 1270, glucuronic acid conjugate of 3-(6-chloro-pyridin-3-ylmethyl)-4-hydroxy-thiazolidine-2-ylidene-cyanamide). WAK 6856 was further oxidized to the respective ketone (PIZ 1297D, 1-(6-chloro-pyridin-3-ylmethyl)-5-oxo-imidazolidin-2-ylidene-cyanamide) which underwent further oxidative cleavage of the methylene bridge (elimination of the Cl-pyridyl moiety) yielding PIZ 1297B (4-oxo-thiazolidine-2-ylidene-cyanamide). Hydroxylation of the cyanamide moiety of YRC 2894 resulted in KNO 1893. The thiazolidine was opened in 2 positions: (1) hydroxylation of the carbon atom in position 5 to yield metabolites PIZ 1253 (1-(6-chloro-pyridin-3-ylmethyl)-3-cyano-1-(2-hydroxyethyl)-2-thiourea) and its respective urea metabolite (WAK 6935, 3-aminocarbonyl-1-(6-chloro-pyridin-3-ylmethyl)-1-(2-hydroxyethyl)-urea) and metabolites PIZ 1250 (N-[(cyanoamino)(methylthio)methylene]-glycine) and PIZ 1241D (N-[aminocarbonyl]amino]thioxomethyl]-glycine) following ring separation (elimination of the Cl-pyridyl moiety) and oxidation of the C-atom in position 5 and/or methylation of the sulfur atom. NTN 35078 (3-(6-chloro-pyridin-3-ylmethyl)-oxazolidin-2-ylidene-cyanamide) formed from PIZ 1253. (2) oxidation and methylation of sulfur in the thiazolidine ring to yield KNO 2672 and the respective urea metabolite KNO 1891 which was further oxidized to metabolites PIZ 1297E (N-(6-chloro-pyridin-3-ylmethyl)-2-methanesulfinyl-acetamide) and PIZ 1269X (N-(6-chloro-pyridin-3-ylmethyl)-2-methanesulfonyl-acetamide). Additionally, oxidative cleavage of the methylene bridge (elimination of the Cl-pyridyl moiety) of YRC 2894 resulted in PIZ 1245 (3H-thiazol-2-ylidene-cyanamide) and the respective urea metabolite PIZ 1249 (thiazol-2-yl-urea). PIZ 1245 was further conjugated with a pentose and sulfate to yield PIZ 1243 (sulfuric acid mono-[5-(2-cyanoimino-thiazol-3-yl)-3,4-dihydroxy-tetrahydro-furan-2-ylmethyl] ester).

At 1 mg/kg, the main metabolite in urine from males was PIZ 1250 accounting for 10.4% of administered dose and for females it was PIZ 1243 at 22.2%. PIZ 1241D (6.1%), PIZ 1243 (5.9%), and PIZ 1249 (5.4%) were the 3 other most abundant metabolites in male urine and PIZ 1250 (9.1%) and PIZ 1249 (5.2%) the 2 in female urine at 1 mg/kg. At 100 mg/kg, the main urinary metabolite was PIZ 1270 at 6.52%. PIZ 1253 (5.7%) and PIZ 1266 (5.6%) were second and third in abundance.

The two main metabolites in feces extracts were WAK 6856 (3.22% (males) and 1.63% (females) at 1 mg/kg and 6.72% at 100 mg/kg) and YRC 2894 (2.21%, 2.22%, and 3.10% respectively). Acceptable. (Green and Leung, 11/16/04)

**\*\*52966-0011** 209474, "[Methylene-<sup>14</sup>C] YRC 2894: General Rat Metabolism Study part B: Toxicokinetics and Metabolism in the Rat", (O. Klein and W. Bornatsch, Bayer AG Crop Protection Development, Institute for Metabolism Research and Residue Analysis, Leverkusen-Bayerwerk, Germany, Bayer Report No. 108116, 5 February 1998). 5 Wistar (Hsd/Win:Wu) rats per sex per group received a single oral gavage dose of <sup>14</sup>C YRC 2894 at 1 or 100 mg/kg (Dose Groups B and D respectively) or a single intravenous dose at 1 mg/kg (Dose group A) or 14 non-radiolabelled YRC 2894 oral gavage doses at 1 mg/kg followed by a single <sup>14</sup>C YRC 2894 oral gavage dose 24 hours later (Dose group C). A separate group of 5 males received a single radiolabelled oral dose at 1 mg/kg followed by measurement of radioactivity in their expired air. Absorption, distribution, and excretion of radioactivity were evaluated over a 48-hour post-dosing period. Metabolites in excreta were identified and quantified. Urine was collected for each animal under cooling with dry ice at the following post-dosing intervals 0-4, 4-8, 8-24, and 24-48 hours. Feces were collected separately for each animal at 0 to 24 hours and 24 to 48 hours post-dosing. Plasma was sampled 0.05, 0.10, 0.20, 0.40, 1, 1.30, 2, 3, 4, 6, 8, 24, 32, and 48 hours after dosing. Expired air was collected from 0-8, 8-24, and 24-48 hours post-dosing.

Maximum mean post-dosing plasma concentrations of radioactivity were reached within 5 minutes after intravenous injections. After oral administration, radioactivity concentrations peaked from 1 to 1.5 hours and from 3 to 4 hours after 1 mg/kg and 100 mg/kg respectively.

After a single oral administration of 1 mg/kg, means of 64.7% and 60.25% of administered radioactivity were excreted in the urine of males and females respectively during 48 hours. For feces, the amounts were 30.11% and 24.7% respectively over the same period. A single intravenous radiolabelled dose at 1 mg/kg resulted in cumulative mean excretion of 68.11% (males) and 61.3% (females) in urine during 48 hours, and 29.29% (males) and 27.48% (females) in feces. After oral dosing with non-radiolabel at 1 mg/kg for 14 days followed by a 1 mg/kg radioactive oral dose, mean urinary excretion was 61.28% and 60.12% for males and females respectively after 48 hours and, in feces, 29.6% and 34.01% respectively. Following a single radiolabelled oral dose at 100 mg/kg, mean percentages excreted in urine were 65.52 (males) and 52.97 (females) over 48 hours. In feces, 39.11% and 9.12% respectively were found. In females at 100 mg/kg, no radiolabel was found in feces after 24 hours, and percentages for males were approximately half those found at 1 mg/kg.

Following oral administration, highest concentrations of radioactivity were found in the skin, gastrointestinal tract (GIT), liver, and kidney at sacrifice (48 hours post-dosing). Concentrations of radioactivity were generally less than 1% of the amount administered except as follows: increased amounts of radioactivity were found in the GIT (>17%), skin (>1%), and liver (>1%) of females that received 100 mg/kg, although the distribution pattern was similar to that of the other groups. Distribution patterns and amounts were similar for oral and intravenous injection routes.

Metabolites in urine and feces were identified and quantified. The main metabolic pathway was the oxidative cleavage of YRC 2894, yielding 6-chloronicotinic acid, which then reacted with glycine to form a hippuric acid type conjugate (WAK 3583), the main metabolite present in urine (not found in feces). The N-nitrile group on YRC 2894 was oxidized to form the amide derivatives KNO 1893 and KNO 1864. The thiazolidine ring was opened (oxidation) to allow formation of 2 sulfoxide compounds, KNO 2672 and KNO 1891. Formation of the oxazole compound NTN 35078 may have resulted from reclosure of one of these. Another biotransformation step was replacement of the chlorine atom on the pyridine ring of chloronicotinic acid by mercaptoacetic acid, KNO 1886. Decarboxylation of this metabolite followed by conjugation with glycine yielded KNO 1889. The acetylcysteine conjugate of the ring-open guanidine compound, KNO 1872, was identified as KNO 2684. Chemical structures for the other metabolites, 6-CNA (not found in feces), KNO 2621/KNO 2665, and WAK 6856, were included. Acceptable. (Green and Leung,

11/16/04).

\*\*52966-0012 209476, “[Methylene-<sup>14</sup>C] YRC 2894: Distribution of the Total Radioactivity in the Rat Determined by Conventional Whole-Body Autoradiography and Radioluminography”, (O. Klein, Bayer AG, Crop Protection Development, Institute for Metabolism Research and Residue Analysis, Leverkusen-Bayerwerk, Federal Republic of Germany, Bayer Report No. 107535, 26 June 1996). 5 male Wistar (Hsd/Win:Wu) rats received a single oral gavage dose of [Methylene-<sup>14</sup>C] YRC 2894 at 5 mg/kg. 1 male received a single intravenous injection at 1 mg/kg. Additionally, another male received a single oral dose of non-labelled YRC 2894 at 5 mg/kg.

Whole-body autoradiography was performed 5 minutes (IV) and 1, 4, 8, 24, and 48 hours after treatment. One animal was used per time point. After sacrifice, each animal was fixed in a stretched position using a metal template and immediately frozen (-70°C). The template was removed and the animal body embedded in a slurry of carboxymethylcellulose (7% to 8%) on the platform of a microtome. 50 µm sections were cut, attached to adhesive tape, and freeze-dried for at least 24 hours. Imaging plates were then exposed to the sections. Exposure times ranged from 15 to 48 hours. After exposure, plates were scanned using an image analyzer and the resulting pictures were printed. The sections were also exposed to conventional X-ray sensitive film at -20°C. Exposure times were between 21 and 134 days. Films were processed and copies of the autoradiograms were prepared using a contact copier and a paper print developer. One animal received a single oral gavage dose of non-radiolabelled YRC 2894 followed by sacrifice and sectioning 4 hours later. Sections were exposed to X-ray sensitive film for 64 days to evaluate possible chemographic effects. None were found.

Radioactivity of the images was quantified. For each organ or partial structure of the organ, defined areas were set and integrated. After background subtraction, the photostimulated luminescence (PSL) per mm<sup>2</sup> was obtained which was in proportion to the equivalent concentration of the radioactivity in that specific tissue. Radioactivity standards of known concentration (Amersham Microscales RPA 504) were coexposed with the rat sections and integrated in the same manner. Radioactivity concentrations in the rat sections were corrected (for tissue consistency (e.g, bone, muscle, fat, etc)), correlated (for water loss during freeze dying), and calculated to obtain radioactivity concentration in microgram per g fresh weight.

Radiograms and radioluminographs were included in the study. Rapid and even distribution of radioactivity to tissues 5 minutes after intravenous injection was noted. Concentration in blood at 5 minutes was lower than in liver, kidney, muscle, preputial gland, adrenals, thyroid, salivary gland, and aorta walls. 1 hour after oral gavage dosing with 5 mg/kg, radioactivity was found in all tissues and organs. Renal excretion had begun. High concentrations of radioactivity were found in liver, adrenals, thyroid, salivary, and preputial glands, skin, aorta walls, and spinal cord. The general pattern of distribution observed 1 hour after dosing was generally maintained throughout the 48 hour investigation period. High renal excretion rates were noted (blackening over kidney) 4 and 8 hours after dosing. At 24 and 48 hours post-dosing, radioactivity was associated with connective tissue in the skin, aorta wall, spinal cord, thyroid, preputial gland, and adrenals. Radioluminographs indicated that > 96% of recovered radioactivity was excreted in urine and feces during 48 hours post-dosing. Acceptable. (Green and Leung, 11/16/04).

#### SUBCHRONIC STUDIES

(Oral)

0041; 209505; “YRC 2894: Pilot Toxicity Study on Rats Acute Oral Toxicity to Non-Fasted Animals Subacute Oral Toxicity with Gavage Administration Over 2 Weeks” (Krötlinger, F., Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer Report No. 107415, 03/15/95). YRC 2894 (Batch no.>NNL 3351-3, purity = 98.3%), mixed in 2% Cremophor EL (v/v) in demineralized water, was administered as an oral gavage dose to a group of non-fasted Hsd/Win:WU rats (an acute oral toxicity study) and daily for 2 weeks to another group of rats, the subacute group, where 3 animals per sex per dose with dose levels of 0, 5, 10, 20, 60, or 120 mg/kg/day were used. For the acute study the following dose levels and number of animals were used- males (5



animals at each dose level): 140, 225, 370, 600, and 700 mg/kg; females: 100, 1000, 2500, and 5000 mg/kg (1 animal at each dose level) and 140, 225, 370, 425, 500, and 600 mg/kg (5 animals at each dose level). During the acute study, mortalities occurred as follows- males: 0/5, 0/5, 0/5, 2/5, 4/5, respectively; females: 0/1, 0/5, 0/5, 1/5, 4/5, 5/5, 5/5, 1/1, 1/1, 1/1, respectively. In the acute study clinical signs were observed at all dose levels; these signs included decreased motility, labored breathing, narrowed palpebral fissure, piloerection, temporary tremor, temporary convulsions, spasmodic state, spastic gait, and lacrimation. In the acute study, necropsy on some of the mortalities revealed dark red lungs, mottled liver, pale spleen, and reddish or clay-colored kidneys while necropsy on the survivors revealed no grossly visible organ lesions. Reported LD<sub>50</sub> (M) = 621 mg/kg, reported LD<sub>50</sub> (F) = 396 mg/kg. Acute NOEL (M/F) not determined. No mortalities occurred in the subacute study. In the subacute study, reduced defecation was observed in all females at 60 and 120 mg/kg/day. Treatment-related decreases in mean body weight and food consumption were observed in both sexes at 60 and 120 mg/kg/day. A treatment-related induction of the following liver enzymes was observed in both sexes at 60 and 120 mg/kg/day: N-demethylase, O-demethylase, Cytochrome P-450, 7-ethoxycoumarin deethylase, aldrin epoxidase, epoxide hydrolase, glutathion-s-transferase, and UDP-glucuronyl transferase. A treatment-related increase in mean relative liver weight in both sexes at 60 and 120 mg/kg/day was observed. **No adverse effects.** NOEL (M/F) = 20 mg/kg/day based on induction of liver enzymes and increase mean relative liver weights.

**Supplemental study** [in the acute study, the animals were not fasted prior to treatment, and in the subacute study, 1) the animals were treated by gavage, 2) the animals were treated for only 2 weeks, 3) no ophthalmological examinations were conducted, and 4) the study report submitted is missing pages 105 through 182, pages that include the histopathology report, and immunotoxicity and cell proliferation data]. (Corlett, 10/14/04)

0076; 209540; "YRC 2894: Study for Subacute Oral Toxicity in Rats (Feeding Study Over 2 Weeks)" (Krötlinger, F., Bayer AG, Fachbereich Toxicology, Wuppertal, Germany, Bayer Report No. 108858-1, 12/09/96). YRC 2894 (Batch no.>NNL 3351-13, purity = 98.60%) was admixed to the diet (containing 1% peanut oil) and fed to 5 Wistar (Hsd Win:WU) rats per sex per dose at dose levels of 0 (untreated diet), 25, 100, 500, or 2000 ppm (0.0, 2.5, 11.2, 49.2, and 187.6 mg/kg/day, respectively for males and 0.0, 2.3, 9.6, 49.5, and 187.2 mg/kg/day, respectively for females) for 2 weeks. No mortalities occurred. Treatment-related constipation was observed in both sexes at 2000 ppm. A treatment-related decrease in body weight was observed in males at 2000 ppm and in females at 500 and 2000 ppm. Treatment-related increases mean gamma-glutamyltransferase, cholesterol, and thyroid stimulating hormone (not statistically significant in males) levels in males and females at 2000 ppm were observed. A treatment-related induction of the following liver enzymes was observed in both sexes at 500 and 2000 ppm: 7-ethoxycoumarin deethylase, aldrin epoxidase, epoxide hydrolase, glutathion-s-transferase, and UDP-glucuronyl transferase. A treatment-related increase in mean relative liver weight in both sexes at 2000 ppm was observed. Necropsy revealed treatment-related distinct lobulation of the liver in males at 2000 ppm. Microscopic examination revealed treatment-related centrilobular hepatocyte hypertrophy with cytoplasmic change in both sexes at 500 and 2000 ppm, an increase in lipid storage in the liver in males at 2000 ppm, and slight hypertrophy in the follicular epithelium of the thyroid in males at 2000 ppm. NOEL (M) = 11.2 mg/kg/day (100 ppm) and NOEL (F) = 9.6 mg/kg/day (100 ppm) based on induction of liver enzymes and centrilobular hepatocyte hypertrophy with cytoplasmic change. **Supplemental study** (only 5 animals per sex per dose level were used, no ophthalmological examinations were conducted, and the animals were treated for only 2 weeks). (Corlett, 08/24/04)

\*\*52966-0084, 0085 209552, 209553, "YRC 2894, Subchronic Range-Finding Study for a Two-Year Study in B6C3F1 Mice (Administration in Feed Over About 14 Weeks)", (U. Wirnitzer and C. Rühl-Fehlert, Bayer AG, Fachbereich Toxikologie, Wuppertal, Germany, Bayer Report No. 106868, 14 March 1995). 10 B6C3F1 mice per sex per group received YRC 2894 (98.6% thiacloprid) in the diet (containing 1% peanut oil) at 0 (basal diet), 50, 250, 1250, and 6250 ppm for 14 weeks. The mean daily intake of YRC 2894 during the treatment period was 19.9, 102.6, 542.4, and 2819.9 mg/kg/day for males and 27.2, 139.1, 704.3, and 3351.0 mg/kg/day for

females at 50, 250, 1250, and 6250 ppm respectively. Group mean male bodyweights were reduced (14%) at 6250 ppm. Female bodyweights were comparable to controls at all treatment levels. Water consumption was reduced in males ( $p \leq 0.01$ ) and females ( $p \leq 0.05$ ) at the high dose level. Group mean relative liver weights were increased at 1250 (M: 9%, F: 10%) and 6250 ppm (M: 39%, F: 42%) for both sexes. No ophthalmology was conducted. Hepatocellular hypertrophy was increased at 250 ppm for males (0 at 0 ppm vs 6 at 250 ppm) and at 1250 (9/M, 10/F) and 6250 ppm (10/M & F) for both sexes. All high dose males showed severity grade 4 (marked); females grades ranged from slight to moderate with no degenerative changes. Increased N-demethylase activity was noted in liver tissue for males at 250 ppm and for both sexes at 1250 and 6250 ppm. P450 content was also increased for both sexes at 1250 and 6250 ppm. There were no toxicologically-relevant changes in hematology or clinical chemistry with dose. A reduction/loss of sex specific vacuolation in the proximal tubules of kidneys was recorded at 1250 and 6250 ppm in males. Record 209553 (Bayer 106868-1) contains additional histopathology of the adrenal glands, female genital tract and mammary glands and an immunohistochemical evaluation of prolactin on the pituitary gland of females at 6250 ppm. In females at 50 ppm and higher, a dose-related increase in the severity/grade of vacuolation of the x-zone of the adrenal cortex leading to hypertrophy was noted. Incidence in the control animals was high (9 at 0 ppm vs 10 at 50 ppm and higher), only the severity/grade increased with dose. In control animals, minimal to mild vacuolation was noted. At 50 ppm and higher vacuolation was increased leading to hypertrophy and enlargement of the x-zone. At 1250 ppm and higher the change was rated as marked or massive. In the ovaries, a reduced number of advanced corpora lutea with eosinophilic cells were recorded at 1250 ppm and higher. No evidence of changes in oviducts, uterus, vagina, mammary gland, or male adrenal glands and no changes were induced in the prolactin-secreting cells in the anterior pituitary. NOEL = 50 ppm (liver changes in males). The significance of the adrenal cortical and ovary changes in females remains unclear since both the adrenals and ovaries depend on complex endocrine regulation mechanisms that were beyond the scope of the study. Acceptable. (Green and Gee, 8/23/04).

0083, 209551; "YRC 2894: Investigations of Subchronic Toxicity in Wistar Rats (Feeding Study Over 12 Weeks with a Subsequent Recovery Period Over 5 Weeks)" (Kröttinger, F. and Geiß, V., Bayer AG, Wuppertal, Germany, Report No. PH-26239, 05/06/97). 821. YRC 2894 (Batch no. NNL 3351-13, purity = 98.6%) was admixed to the diet (containing 1% peanut oil) and fed to 10 Wistar rats per sex per dose at dose levels of 0, 25, 100, 400, or 1600 ppm (0, 1.9, 7.3, 28.6, and 123.2 mg/kg/day, respectively for males and 0, 2.0, 7.6, 35.6, and 160.6 mg/kg/day, respectively for females) for up to 13 weeks [with 10 additional rats per sex per dose level at 0 and 1600 ppm dose levels to test recovery (5-week recovery period used)]. No mortalities occurred. No dose-related clinical signs were observed. A treatment-related decrease in mean body weight was observed in both sexes at 1600 ppm with recovery observed in the males but not the females. A treatment-related increase the mean cholesterol level in males at 1600 ppm and in females at 400 and 1600 ppm with partial recovery observed in females but not males. A treatment-related induction of the following liver enzymes was observed in both sexes at 400 and 1600 ppm: N-demethylase, O-demethylase, Cytochrome P-450, 7-ethoxycoumarin deethylase, 7-ethoxyresorufin deethylase, aldrin epoxidase, epoxide hydrolase, glutathion-s-transferase, and UDP-glucuronyl transferase with all parameters returning to background levels in the recovery group animals of both sexes. A treatment-related increase in mean relative liver weight in both sexes at 1600 ppm was observed with recovery observed in the males but not the females. Microscopic examination revealed treatment-related hepatocellular hypertrophy and cytoplasmic (perinuclear) change (fine granular to vesicular structure) in the liver in both sexes at 400 and 1600 ppm; no hepatocellular hypertrophy was observed in the recovery group females and it was reduced in recovery group males and no cytoplasmic change was observed in recovery group animals of either sex. **No adverse effects.** NOEL (M) = 7.3 mg/kg/day (100 ppm) and NOEL (F) = 7.6 mg/kg/day (100 ppm) based on induction of liver enzymes and hepatocellular hypertrophy with cytoplasmic change. **Acceptable.** (Corlett, 09/10/04)

0075; 209539; "YRC 2894: Pilot Study on Subacute Toxicity in B6C3F<sub>1</sub> Mice (Administration in Feed Over 3 Weeks)" (Wirnitzer, U., Bayer AG, Institute for Industrial

Toxicology, Wuppertal, Germany, Bayer Report No. 108859, 11/04/94). YRC 2894 (Batch no. NLL 3351-13, purity = 98.60%) was admixed to the diet (containing 1% peanut oil) and fed to 3 B6C3F<sub>1</sub> mice per sex per dose at dose levels of 0 (untreated diet), 100, 1000, or 10000 ppm (0.0, 30.1, 367.8, and 4141.0 mg/kg/day, respectively for males and 0.0, 63.9, 559.3, and 5785.1 mg/kg/day, respectively for females) continuously for 3 weeks. No mortalities occurred. No clinical signs were observed. A treatment-related increase in mean relative liver weight (not statistically significant) in males and females at 1000 and 10000 ppm was observed. Necropsy revealed 2 of 3 males with enlarged livers. NOEL (M) = 30.1 mg/kg/day (100 ppm) and NOEL (F) = 63.9 mg/kg/day (100 ppm) based on increased mean relative liver weight (not statistically significant). **Supplemental study** (only 3 animals per sex per dose level were used, no clinical chemistry was performed, no histopathology was performed, and the animals were treated for only 3 weeks). (Corlett, 08/19/04)

0082; 209550; "YRC 2894: Study for Subacute Oral Toxicity in Mice (Feeding Study Over 2 Weeks)" (Krötlinger, F., Bayer AG, Fachbereich Toxicology, Wuppertal, Germany, Bayer Report No. 108864, 02/25/97). YRC 2894 (Batch no. NLL 3351-13, purity = 98.60%) was admixed to the diet (containing 1% peanut oil) and fed to 5 B6C3F<sub>1</sub>/Bom mice per sex per dose at dose levels of 0 (untreated diet), 50, 200, 2000, or 10000 ppm (0.0, 21.6, 84.3, 765.1, and 4143.2 mg/kg/day, respectively for males and 0.0, 29.8, 113.2, 1201.2, and 5449.8 mg/kg/day, respectively for females) for 2 weeks. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. Serum chemistry revealed no treatment-related effects. Treatment-related inductions of the following liver enzymes were observed: 7-ethoxycoumarin deethylase, 7-ethoxyresorufin deethylase, and aldrin epoxidase in males at 2000 ppm and above and in females at 200 ppm and above; epoxide hydrolase in both sexes at 10000 ppm; glutathion-s-transferase in both sexes at 200 ppm and above; UDP-glucuronyl transferase in both sexes at 2000 ppm and above. A treatment-related increase in mean relative liver weight in both sexes at 2000 and 10000 ppm (not statistically significant in males at 2000 ppm) was observed. Microscopic examination revealed treatment-related centrilobular hepatocyte hypertrophy with more amorphous cytoplasm in males at 200 ppm and above and in females at 2000 ppm and above. NOEL (M) = 21.6 mg/kg/day (50 ppm) and NOEL (F) = 29.8 mg/kg/day (50 ppm) based on induction of liver enzymes and centrilobular hepatocyte hypertrophy with cytoplasmic change. **Supplemental study** (only 5 animals per sex per dose level were used, no ophthalmological examinations were conducted, and the animals were treated for only 2 weeks). (Corlett, 08/27/04)

52966-0080 209548, "YRC 2894, Subacute Toxicity Study in Beagle Dogs (Dose Range Finding Study by Feed Admixture Over at Least 10 Weeks)", (H. Wetzig and V. Geiß, Bayer AG, Institute for Toxicology, Wuppertal, Germany, Study No. T 8 055 594, Report No. 27177 A, 11 February 1999). 2 Beagle dogs per sex received YRC 2894 (98.6% thiacloprid) in the diet at 0 (basal diet), 100, 300, 1000 ppm (increased to 1250 ppm from day 19, to 1600 ppm from day 26, and to 2500 ppm from day 38) for up to 10 weeks. An additional group received 2500 ppm from day 38 through 66. The group mean YRC 2894 consumption for both sexes was 3.3, 9.6, 80.0, and 65.7 mg/kg/day at 100, 300, 1000 - 2500, and the additional 2500 ppm group respectively at week 9. Reduced food consumption and bodyweight were observed in females of the added 2500 ppm group during the 4 week treatment period. Urea and creatinine values were increased at 1000-2500 and 2500 ppm. Alanine aminotransferase values were slightly increased for males in the 2500 ppm group. Thyroxine (T4) was reduced and triiodothyronine (T3) was increased in 2500 ppm females. Marginal changes were identified in liver enzymes. O-Demethylase values were slightly increased in 2500 ppm group animals. 7-ethoxycoumarin deethylase (ECOD), epoxide hydrolase (EH), and glutathione-S-transferase (GS-T) were slightly increased in the 1000-2500 and 2500 ppm females. Absolute and relative prostate weights were increased for 1000-2500 and 2500 ppm males. The increase was greater at 1000-2500 ppm probably due to longer time on treatment. Microscopy revealed slight cytoplasmic change in liver cells of 1 female at 1000-2500 ppm and 1 per sex at added 2500 ppm. NOEL = 300 ppm (9.6 mg/kg/day) based on increased prostate weights, liver histopathology, and clinical chemistry. Not a guideline study. Supplemental. (Green and Gee, 10/21/04).

**52966-0051 209515**, "Subchronic Toxicity Study in Beagle Dogs (Feeding Study for About 15 Weeks)", (H. Wetzig and M. Rinke, Bayer AG, Institute for Toxicology, Wuppertal, Germany, Study No. T 0 058 331, Bayer Report No. 108350, 8 May 1998). 4 Beagle dogs per sex per group received YRC 2894 (96.8% thiacloprid) in the diet at 0 (basal diet), 250, 1000, and 2000 ppm for 15 weeks. High dose animals received treated feed for 13 weeks (days 1-4 at 4000 ppm, days 5-14 at 0, and day 15 to necropsy at 2000 ppm). Dosage was reduced due to vomiting, reduced feed intake, and refusal to eat. The group mean (both sexes) intakes of YRC 2894 during weeks 1 through 15 were 8.7, 34.7, and 66.7 mg/kg/day at 250, 1000, and 2000 ppm respectively. Slight reductions (NS) in food consumption (both sexes) and male bodyweight were recorded at the high dose level. Thyroxine (T4) was decreased at the mid and high dose levels. Absolute and relative prostate weights were increased at the mid and high dose levels and absolute and relative liver weights were higher in all treated groups (both sexes) relative to controls. Histopathology revealed slight to moderate hypertrophy of the prostate in all males at the mid and high dose levels. Additionally, at the high dose level, an increase in the number of degenerated spermatocytes was noted in the testes (control: 1/4 vs high dose: 2/4) and epididymides (control: 1/4 vs high dose: 4/4). Leydig cells appeared slightly more prominent at the high dose level (control: 1/4 vs high dose: 3/4). The significance of these changes is unclear given the wide variation in severity and incidence found in young mature dogs. Peak mean plasma levels (6 hours post-treatment) of YRC 2894 at week 14 indicated a high absorption rate (2, 6, and 14 µg/ml at the low, mid, and high dose levels respectively). NOEL = 250 ppm (8.7 mg/kg/day) based on increased prostate weight and hypertrophy. **Possible adverse effect:** prostate enlargement (hypertrophy). Acceptable. (Green and Gee, 8/19/04)

(Dermal)

0050; 209514; "YRC 2894: Study for Subacute Dermal Toxicity in Rats (Four-Week Treatment and Two-Week Recovery Period)" (Krotlinger, F. and Sanders, E., Bayer AG, Department of Toxicology, Wuppertal, Germany, Study No. T3060007, Bayer Report No. 107625, 01/30/97). 822. YRC 2894 (Mixed batch no. 290894, purity = 97.20%) was moistened with tap water and applied to the shaved dorsal skin of 5 HsdCpb:WU rats per sex per dose at dose levels of 0 (tap water only), 100, 300, or 1000 mg/kg/day for 6 hours per day 5 days per week for 4 weeks (7 days per week during the 4<sup>th</sup> week) [with 5 additional rats per sex per dose level at the 0 and 1000 mg/kg/day dose levels to test recovery (2-week recovery period used)]. No mortalities occurred. No treatment-related clinical signs were observed. No skin irritation was observed at the treatment sites. No effect on body weight was observed. Hematological and clinical chemistry investigations revealed no treatment-related effects. A treatment-related increase in mean relative liver weight in both sexes at 1000 mg/kg/day was observed with recovery observed in both sexes. Macroscopic examination revealed no treatment-related abnormalities. Microscopic examination revealed treatment-related hepatocellular centrilobular hypertrophy in males at 300 and 1000 mg/kg/day and in females at 1000 mg/kg/day; no hepatocellular centrilobular hypertrophy was observed in the recovery group females and it was reduced in recovery group males. **No adverse effects.** NOEL (M, systemic) = 100 mg/kg/day and NOEL (F, systemic) = 300 mg/kg/day based on centrilobular hepatocellular hypertrophy. NOEL (M/F, skin) = 1000 mg/kg/day based on no treatment-related effects at the highest dose tested. **Acceptable.** (Corlett, 10/13/04)

(Inhalation)

0079; 209543; "Pilot Study on Subacute Inhalation Toxicity in Rats (Exposure: 5 x 6 Hours)" (Pauluhn, J., Bayer AG, Department of Toxicology, Wuppertal, Germany, Study No. T0058313, Bayer Report No. 107024, 08/21/95). YRC 2894 (Mixed Batch No. 290894, purity = 97.2%) was aerosolized and administered in nose-only manner to 10 Wistar Hsd Win:WU rats per sex per dose at dose levels (mean gravimetric concentration) of 0 (conditioned air), 0.00197, 0.019, or 0.205 mg/l (with mean MMAD (GSD) of -, 3.3 (2.3), 2.9 (2.1), 3.3 (1.8), respectively) for 6 hours per day, 5 days per week for 1 week at which point 5 animals per sex per dose were sacrificed (day 7); the remaining 5 animals per sex per dose were observed for additional 2 weeks prior to sacrifice (day 21). No mortalities occurred. Piloerection, bradypnea, labored

breathing pattern, reduced motility, tremor, flaccid appearance, and mydriasis were observed in both sexes at 0.205 mg/l after exposure to the test material with all clinical signs clearing 3 days after the cessation of treatment. A treatment-related decrease in mean body weight was observed in both sexes at 0.205 mg/l on days 4 and 7 with reversibility of this effect demonstrated by day 14. A treatment-related induction of the following liver parameters was observed in animals of both sexes at 0.205 mg/l sacrificed on day 7: N-demethylase, O-demethylase, cytochrome P-450, and triglycerides; these effects were not observed in animals sacrificed on day 21. In animals sacrificed on day 7, a treatment-related decrease in mean relative thymus weight and a treatment-related increase in mean relative liver weight in both sexes at 0.205 mg/l were observed; no treatment-related organ weight effects were observed in the animals sacrificed on day 21. Necropsy on animals sacrificed on day 7 revealed treatment related black spleens in females at 0.019 and 0.205 mg/l and small thymus and enlarged liver in males at 0.205 mg/l; no macroscopic treatment-related effects were observed in animals sacrificed on day 21. **No adverse effects.** NOEL (M/F) = 0.019 mg/l based on a decrease in mean relative thymus weight (males), black spleens (females), an increase in mean relative liver weight (both sexes), and an induction of liver enzymes (both sexes). **Supplemental study** (1) the animals were treated for only 1 week, 2) no histopathology was performed on the test animals, 3) no ophthalmological examinations were conducted on the test animals, and 4) particle size analysis was not conducted daily). (Corlett, 09/29/04)

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#### Neurotoxicity

0042; 209506; “**Original:** An Acute Oral Neurotoxicity Screening Study with Technical Grade YRC 2894 in Fischer 344 Rats; **Supplemental:** A Special Acute Oral Neurotoxicity Study to Establish a No-Observed-Effect Level with Technical Grade YRC 2894 in Fischer 344 Rats” (Sheets, L.P., Bayer Corporation, Agricultural Division, Toxicology, Stilwell, KS, Agricultural Division Report No. 107633-1, 05/04/98). Technical Grade YRC 2894 (Mixed Batch No. 290894, purity = 96.8-97%) in 0.5% methylcellulose-0.4% Tween 80 in deionized water was administered by gavage in a single dose to 12 Fischer 344 CDF(F-344)/BR rats per sex per dose at dose levels 0 (vehicle only), 3.1, and 11 mg/kg. The animals were observed for 1 day following treatment. No mortalities occurred. No treatment-related clinical signs were observed. FOB conducted on the day of treatment (at the time of peak neurobehavioral effects) revealed no treatment-related effects in either sex. Treatment-related decreases in mean motor and in mean locomotor activity (not statistically significant) were observed in females at 11 mg/kg. **No adverse effects.** NOEL (M) = 11 mg/kg based on no effects at the highest dose tested and NOEL (F) = 3.1 mg/kg based on decreased motor and locomotor activity. **Supplemental study** (only 2 dose levels were used, the animals were observed for only 1 day after treatment, FOB was conducted only at the time of peak neurobehavioral effects and not prior to treatment, and no necropsy and histopathology were performed). (Corlett, 09/14/04)

0049; 209513; “A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade YRC 2894 in Fischer 344 Rats” (Sheets, L.P., Bayer Corporation, Agricultural Division, Toxicology, Stilwell, KS, Agricultural Division Report No. 107619, 06/03/97). 827. Technical grade YRC 2894 (Mixed Batch No. 290894, purity = 97.5%) was admixed to the feed (containing 1% corn oil by weight) and fed to 12 Fischer 344 rats per sex per dose at dose levels of 0 (untreated diet), 50, 400, or 1600 ppm (0, 2.94, 24.2, and 101 mg/kg/day, respectively for males and 0, 3.41, 27.9, and 115 mg/kg/day, respectively for females) for 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. A treatment-related decrease in mean body weight was observed in males at 1600 ppm. A treatment-related decrease in mean food consumption was observed in both sexes at 400 and 1600 ppm. FOB assessments revealed no treatment-related effects. Motor activity assessments revealed no treatment-related effects. Macroscopic and neuropathological examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M) = 2.94 mg/kg/day (50 ppm) and NOEL (F) = 3.41 mg/kg/day (50 ppm) based on decreased mean food consumption. **Acceptable.** (Corlett and Leung, 09/22/04)